Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 5729–5733, June 1995
Medical Sciences

The carboxyl-terminal domain of the p53 protein regulates sequence-specific DNA binding through its nonspecific nucleic acid-binding activity

J. HENRI BAYLE, BRIAN ELENBAAS, AND ARNOLD J. LEVINE

Department of Molecular Biology, Lewis Thomas Laboratory, Princeton University, Princeton, NJ 08544

Contributed by Arnold J. Levine, February 28, 1995

ABSTRACT **The murine p53 protein contains two nucleic acid-binding sites, a sequence-specific DNA-binding region localized between amino acid residues 102–290 and a nucleic acid-binding site without sequence specificity that has been localized to residues 364–390. Alternative splicing of mRNA generates two forms of this p53 protein. The normal, or majority, splice form (NSp53) retains its carboxyl-terminal sequence-nonspecific nucleic acid-binding site, which can negatively regulate the sequence-specific DNA-binding site. The alternative splice form of p53 (ASp53) replaces amino acid residues 364–390 with 17 different amino acids. This protein fails to bind nucleic acids nonspecifically and is constitutive for sequence-specific DNA binding. Thus, the binding of nucleic acids at the carboxyl terminus regulates sequence-specific DNA binding by p53. The implications of these findings for the activation of p53 transcriptional activity following DNA damage are discussed.**

The p53 protein has been structurally and functionally divided into four domains (see Fig. 1 *Upper*). The amino-terminal 42 amino acid residues encompass a transcriptional activation region that interacts with several components of the basal transcriptional machinery of the cell (1–4). Amino acid residues 102–290 form a large central domain of the protein (5–8) that binds to DNA in a sequence-specific fashion. DNA elements that are specifically bound by the p53 protein are found in genes regulated by the p53 transcription factor (9–18). The third domain of the p53 protein is localized between amino acid residues 320 and 360 and mediates the formation of p53 tetramers in solution (8, 19–22). The fourth domain resides between residues 364 and 390 in the murine p53 protein (367 to 393 in the human protein) and contains a set of residues that are necessary to bind to nucleic acids in a sequence-independent fashion and promote the formation of double-stranded DNA or RNA from single-strand complementary nucleic acids (23–25). This fourth domain has been implicated in the regulation of sequence-specific DNA binding of the p53 protein, which occurs through the central domain of the protein. The monoclonal antibody PAb 421, which binds to amino acid residues 370–378 in this fourth domain, enhances p53 sequence-specific DNA binding (26, 27) by the second domain. Similarly, the deletion of the last 30 amino acids from the human p53 protein also enhances sequence-specific DNA binding by the second domain (26).

A second form of the p53 transcript utilizing an alternative splice acceptor site within intron 9 of the p53 gene has been demonstrated in a variety of murine cell types and tissues (28, 29), and the p53 protein encoded by this transcript has been shown to be present in a murine 3T3 cell line (30). This alternative splice form (ASp53) lacks the basic carboxyl-terminal 26 amino acids of the normal splice form (NSp53) and replaces them with 17 amino acids encoded from intron 9 prior to a translational termination codon (Fig. 1 *Lower*). The carboxyl termini of human p53 and murine NSp53 are highly homologous. Because the final 30 amino acids of the human and murine p53 proteins appear to be critical in the regulation of sequence-specific DNA binding (26, 31), it was of considerable interest to test the relative abilities of the murine ASp53 and NSp53 to bind to p53-specific DNA elements in the presence or absence of nonspecific-sequence DNA.

MATERIALS AND METHODS

Preparation of Nuclear Extracts and Gel Mobility-Shift Assays. Nuclear extracts and whole-cell extracts were prepared essentially as described by Lassar *et al.* (32). DNA-binding reactions were performed with 3 μl of extract in a volume of 30 μl containing 20 mM Hepes (pH 7.6), 1.5 mM $MgCl_2$, 1 mM dithiothreitol, 100 mM NaCl, 7% glycerol, 1.2 μg of dI-dC, and 0.1–0.4 ng of ^{32}P-labeled double-stranded oligonucleotide of the sequence 5′-TTAAGGACATGCCCGGGCATGTCC-3′. Nonspecific competitor oligonucleotide was of the sequence 5′-TGTAGAGCTTAAGCTCTACCATCTCGAATTCGAGATGT-3′. Monoclonal antibodies were purified over a column of protein A-Sepharose (Sigma) and eluted with weak acid. DNA–protein complexes were resolved by native gel electrophoresis through 4% polyacrylamide gels in 0.5X TBE (0.045 M Tris borate, pH 7.6/0.001 M EDTA) at 200 V.

Preparation and Purification of Polyclonal Antiserum to ASp53. A synthetic peptide corresponding to the amino acid sequence of the carboxyl-terminal 17 amino acids of ASp53 (Fig. 1 *Lower*) was conjugated to a helper protein and used to immunize New Zealand White rabbits. Antibodies were purified from the antiserum by passage over a column of Immunoadsorbent beads (Boehringer Mannheim) to which the antigenic peptide was linked according to the manufacturer's instructions. Antibodies were eluted in fractions with 100 mM glycine (pH 2.5) and immediately neutralized to give a final solution of 200 mM Tris/glycine (pH 7.1) containing 150 mM NaCl and stored at 4°C.

Protein Purification. Murine wild-type p53 was purified essentially as described by Wu *et al.* (23) except that an immunoaffinity column of PAb 421 was used to purify NSp53 with elution by its epitope peptide. ASp53 was purified over a column of purified anti-AS antiserum conjugated to protein A-Sepharose (Sigma) with dimethylpimilimidic acid as described by Harlow and Lane (35) and eluted with the epitope peptide for ASp53.

RESULTS

To compare the activities of NSp53 and ASp53 cell lines were created that express only ASp53 or NSp53. The 10 (1) cell line is an immortalized murine embryo fibroblast line that lacks

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NSp53 and ASp53, normal and alternative splice form of p53, respectively.

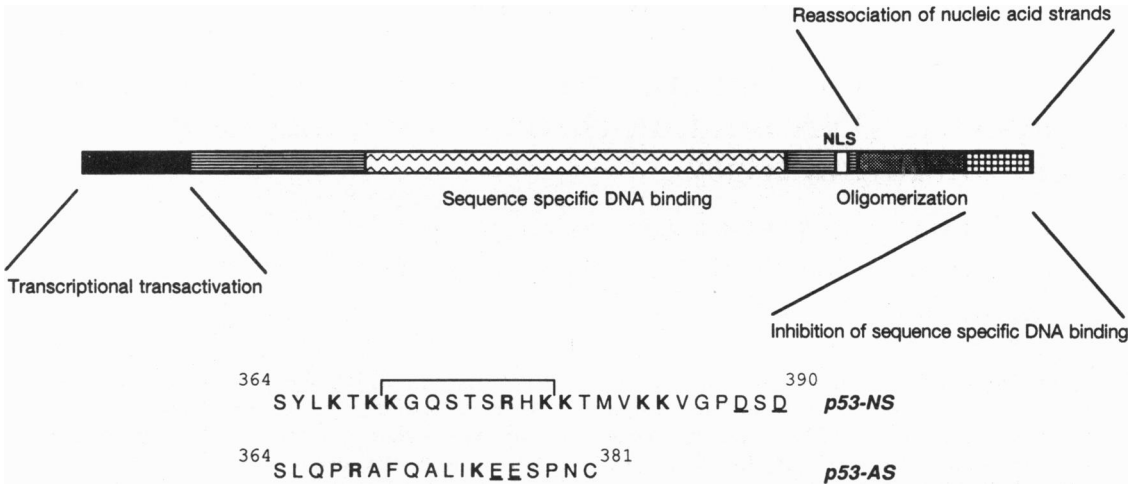

FIG. 1. Domain structure of the p53 protein. (*Upper*) The structure of the p53 protein has been functionally mapped into several domains. The amino terminus of the protein is necessary and sufficient to activate transcription when tethered to DNA near a promoter element. A large, central DNA-binding domain mediates the specific binding of the p53 protein to discrete DNA recognition elements. Sequences surrounding amino acid 312 of murine p53 form the major nuclear localization signal (NLS) for the protein (33). The p53 protein binds to DNA as a tetramer via a carboxyl-terminal oligomerization domain. The oligomerization domain and the extreme carboxyl terminus of the protein mediate a nonspecific DNA-binding activity and the ability of the protein to catalyze the association of single strands of nucleic acids. The extreme carboxyl terminus from residue 364 to residue 390 is essential for the inhibition of sequence-specific DNA binding. This region is changed by alternative splicing of the p53 transcript. (*Lower*) Alternate splice forms of the murine p53 protein. Murine p53 can exist in one of two splice forms differing in the sequence of the extreme carboxyl terminus beginning at amino acid 364. The sequence of the carboxyl terminus of NSp53 (or p53-NS) and ASp53 (or p53-AS) is depicted in the one-letter amino acid code. Basic residues are shown in boldface type, and acidic residues are underlined. The bracket above amino acids 370–378 of NSp53 indicates the epitope for monoclonal antibody PAb 421 (34), which can enhance the sequence-specific DNA binding of NSp53.

endogenous p53 expression (36). 10 (1) VAS 5 cells express a cDNA encoding a temperature-sensitive mutant of ASp53 (amino acid 135 is changed from alanine to valine). 10 (1) VAL 5 cells express only the NSp53 form of p53 using the same temperature-sensitive mutant and the same cellular background (17). Nuclear extracts of these two cell lines, which had been incubated at the permissive temperature of 32°C, yield p53 protein in the wild-type conformation. The parental 10 (1) cell line also was used to prepare nuclear extracts not containing any p53 protein. These extracts were incubated with a DNA oligonucleotide containing the p53 consensus DNA-binding site (16) (Fig. 2 *Upper*). No specific DNA-binding activity could be observed in extracts from 10 (1) cells that lack p53 protein. 10 (1) VAS 5 nuclear extracts contained a specific DNA-binding activity mediated by ASp53 which could be competitively inhibited by an excess of unlabeled oligonucleotide encoding the p53 consensus site but not by an unrelated oligonucleotide. This activity was supershifted by incubation with purified monoclonal antibody PAb 248, which recognizes an amino-terminal epitope of the murine p53 protein (34, 37), but not by monoclonal antibody PAb 421, which does not recognize ASp53. Inclusion of purified rabbit polyclonal antiserum specific for ASp53 in the DNA-binding reaction created a supershifted complex that was more diffuse than that of monoclonal antibody PAb 248, presumably because of the heterogeneous nature of polyclonal antisera. A striking contrast was observed in DNA-binding reactions with 10 (1) VAL 5 cell nuclear extract that contain only NSp53. No sequence-specific DNA binding was observed unless the binding reaction was incubated with the carboxyl-terminal-specific antibody PAb 421 (34, 38). This binding activity was specifically inhibited by the p53 consensus oligonucleotide. It is significant that no similar increase in the intensity of DNA binding by ASp53 was observed by addition of the antiserum even though the polyclonal antiserum recognizes a carboxyl-terminal epitope of ASp53 (as does the PAb 421 antibody with NSp53).

Similar results were obtained when gel mobility-shift assays were performed with whole-cell extracts of insect cells infected with a recombinant baculovirus expressing either wild-type murine NSp53 or ASp53 (Fig. 2 *Lower*). Specific binding by recombinant ASp53 was observed which could be supershifted by PAb 248 or by anti-ASp53 antisera but not by PAb 421. DNA binding by NSp53 was not observed unless PAb 421 was included in the reaction to activate sequence-specific DNA binding. It should be noted that in all of these experiments, the binding of the p53 protein to sequence-specific DNA is carried out in crude extracts where nonspecific DNA and RNA can compete at both the specific and nonspecific binding sites.

These results confirm and extend earlier observations showing that an inhibitory activity of the sequence-specific DNA binding was mediated by the extreme carboxyl terminus of the human p53 protein, and this could be overcome by binding of the 421 antibody (26, 27), by deletion of the carboxyl-terminal 30 amino acids (26), or by ASp53 (31). In this study the replacement of the carboxyl-terminal 26 amino acids of murine NSp53 with 17 different amino acids by alternative splicing produced an ASp53 protein that was constitutively active for DNA binding (i.e., not regulated or inhibited) and was not further activated by antibody binding to the carboxyl terminus. These results argue that the inhibitory or regulated activity was intrinsic to the sequences found in the carboxyl terminus of NSp53.

The nonspecific DNA binding activity was previously localized to the carboxyl-terminal domain of p53 (7, 23). It seemed likely that basic residues in the extreme carboxyl terminus of NSp53 mediate this nonspecific DNA binding and that this nonspecific DNA binding may be essential for the carboxyl terminus of NSp53 to inhibit sequence-specific DNA binding. To test this hypothesis, ASp53 and NSp53 were purified from the appropriate baculovirus-infected insect cells by immunoaffinity chromatography. Gel-shift DNA-binding reactions were performed with these purified proteins in the absence or the presence of increasing amounts of nonspecific DNA (Fig. 3). The binding of sequence-specific DNA to purified ASp53 was resistant to competition by even a 10,000-fold excess (by mass) of nonspecific DNA. By contrast, NSp53 was effectively inhibited for binding to its consensus oligonucleotide by a relatively small excess (about 80-fold) of nonspecific DNA. These results demonstrate that nonspecific DNA can act as a

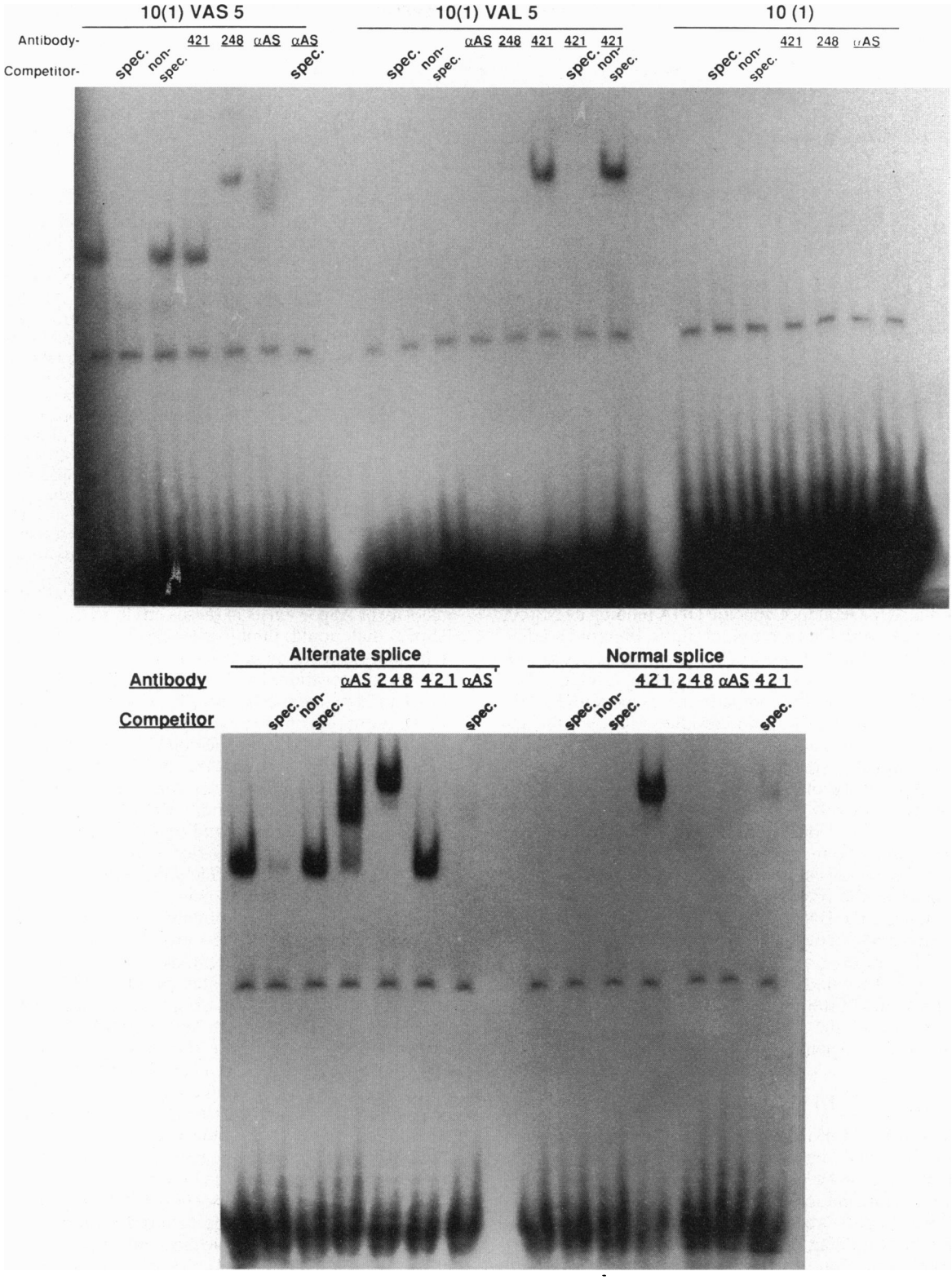

FIG. 2. ASp53 is constitutive for sequence-specific DNA binding, whereas NSp53 must be activated by antibody binding to its carboxyl terminus. (*Upper*) DNA-binding assays were performed with nuclear extracts prepared from cells expressing only alternative splice p53 [10 (1) VAS 5], the normal splice form [10 (1) VAL 5], or no p53 [10 (1)]. Nuclear extracts were incubated with ^{32}P-labeled DNA oligonucleotides containing 100 pg of the p53 consensus DNA-binding site (16) and a large excess (1 μg) of unlabeled deoxyinosine-deoxyguanosine (dI-dC). Excess unlabeled competitor oligonucleotides containing the specific recognition sequence for p53 (spec.) or an unrelated sequence (non-spec.) were included as indicated above the lanes. Antibodies (0.8–1 μg) specific for the carboxyl terminus of NSp53 (lane 421) and of ASp53 (lane αAS) or an amino-terminal epitope on both splice products (lane 248) were included as indicated to supershift p53–DNA complexes. Protein complexed to DNA is visualized as a shift in the mobility of the labeled DNA following native gel electrophoresis. (*Lower*) Gel mobility-shift assays performed with whole-cell extracts from insect cells infected with recombinant baculovirus expressing ASp53 or NSp53. Specific and nonspecific competitor oligonucleotides and purified antibodies were included as indicated.

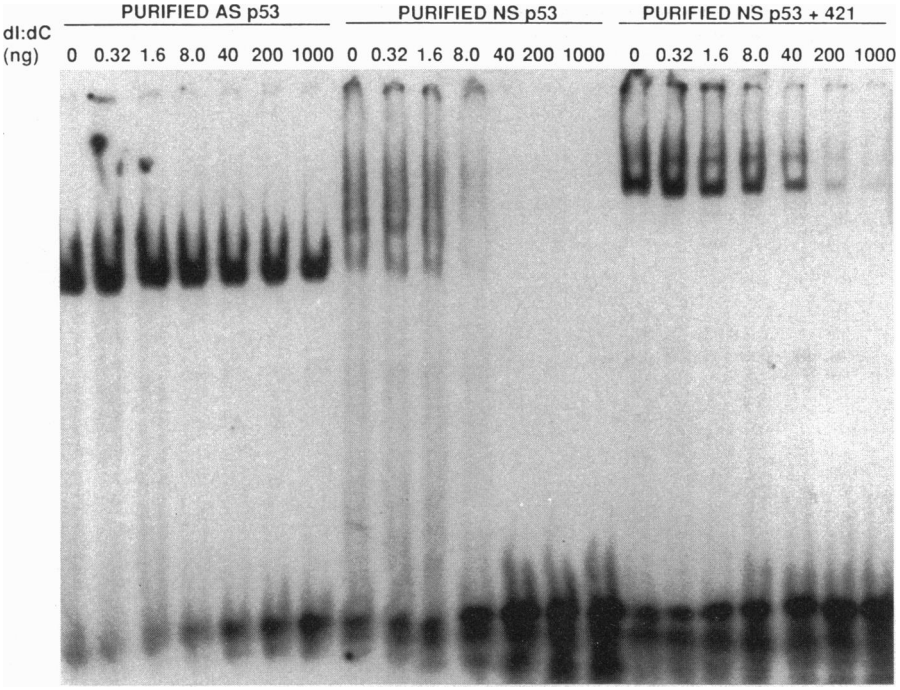

FIG. 3. Inhibition of sequence-specific DNA binding by the carboxyl terminus of NSp53 is mediated by nonspecific DNA binding. ASp53 (*Left*) and NSp53 (*Center*) protein was purified from baculovirus-infected insect cells by immunoaffinity chromatography. Purified p53 (100 ng) was incubated with labeled p53 consensus oligonucleotide (100 pg) and increasing amounts of unlabeled dI-dC as indicated. (*Right*) PAb 421 (2.8 μg) was included with NSp53 to activate specific DNA binding at high concentrations of dI-dC. DNA–protein complexes were analyzed by gel mobility-shift assay.

noncompetitive inhibitor (not at the sequence-specific DNA binding domain) for sequence-specific DNA binding by NSp53 via an interaction with the carboxyl terminus. Previous studies have suffered from the ambiguity of being unable to determine whether the apparent sensitivity of sequence-specific DNA binding by p53 to nonspecific competition was mediated at the central p53 DNA-binding domain (residues 102–290, the sequence-specific binding domain) or at the carboxyl-terminal nonspecific binding site (residues 364–393). If the former were correct, the specificity of p53 for specific DNA sequences would be weak and easily blocked by competition. Indeed, as the concentration of dI-dC approaches 80-fold excess to the specific DNA probe bound to the NSp53 protein (Fig. 3), one can begin to observe this kind of competition at the second or central domain of the p53 protein. It is clear, however, that binding of nonspecific DNA at the carboxyl-terminal domain regulates, either allosterically or by direct interaction (sterically), the ability of the central domain to bind to p53-specific DNA elements. The binding of nucleic acids to the last 26 amino acid residues of the p53 protein thus appears to control the conformation of the protein and its ability to act as a transcription factor dependent upon DNA binding.

DISCUSSION

The results presented in this report provide a mechanism to explain the observed regulation by NSp53 for site-specific DNA binding (Fig. 4*A*). Binding to nonspecific DNA locks NSp53 into a conformation that cannot bind to its sequence-specific DNA target. Abrogation of the carboxyl-terminal nonspecific DNA-binding by contact with PAb 421 antibody results in the conversion of NSp53 into a form that can bind specifically to its DNA-binding elements. In this regard, it is interesting that the epitope for PAb 421 overlaps several of the basic amino acids in the carboxyl terminus of NSp53 that may be expected to mediate an interaction with DNA. Such a model predicts that in the cell nucleus, a large fraction of NSp53 molecules may be bound nonspecifically to DNA and are blocked for sequence-specific DNA binding and transcriptional activation. By contrast, ASp53 appears to be constitutive for site-specific DNA binding because it lacks the carboxyl-terminal nonspecific DNA-binding activity. It should be noted, however, that ASp53 is present at far lower levels than NSp53 in all cell lines tested to date (29–31) and that the great majority of ASp53 exists in these cells as heterooligomers with NSp53 that could inhibit site-specific DNA binding in a dominant-negative manner (31).

These observations may have important implications toward understanding the mechanisms by which p53 may be activated for transcription. p53-responsive genes are transcriptionally activated after DNA damage coincident with increases in the level of the p53 protein (18, 39–41). It may be that the conformational regulation of sequence-specific binding by NSp53 could be an important regulatory component in the response to DNA damage and that nonspecific DNA binding by the carboxyl terminus could mediate this regulation as a part of the mechanism by which p53 transcriptional activity is induced following DNA damage.

It is intriguing that the human p53 protein and a carboxyl-terminal fragment of the p53 protein have been observed to localize to single-stranded regions of DNA and to the termini of nonspecific DNA templates (S. Lee, B.E., A.J.L., and J. Griffith, unpublished observations). These substrates are similar to the lesions produced following DNA damage. A greater affinity of the carboxyl-terminal nonspecific DNA-binding activity for sites of DNA damage suggests that the carboxyl-terminal DNA-binding domain may serve to scan for and recognize DNA damage directly. A consequence of such a recognition would be to colocalize p53 to regions with other factors important in damage recognition and repair (Fig. 4*B*). One or more of these factors may be hypothesized to activate p53. An inactivation of nonspecific DNA binding via addition of acidic groups to the basic carboxyl terminus of NSp53 by phosphorylation is a plausible mechanism to promote a change in p53 from a latent to an active form for site-specific DNA binding. Consistent with this idea, it has been reported that addition of purified casein kinase II, which phosphorylated the penultimate serine of NSp53 *in vitro* (42), to DNA-binding reactions activates p53 for site-specific DNA binding (26). Alternatively, protein–protein interaction at the carboxyl-terminus of NSp53 following DNA damage may activate p53 in a manner similar to the binding of PAb 421 *in vitro*.

The markedly increased DNA binding activity of ASp53 relative to NSp53 implies that a potential mechanism for activating p53 function within a cell could be by increasing the relative amount of ASp53 to NSp53. It has been reported that

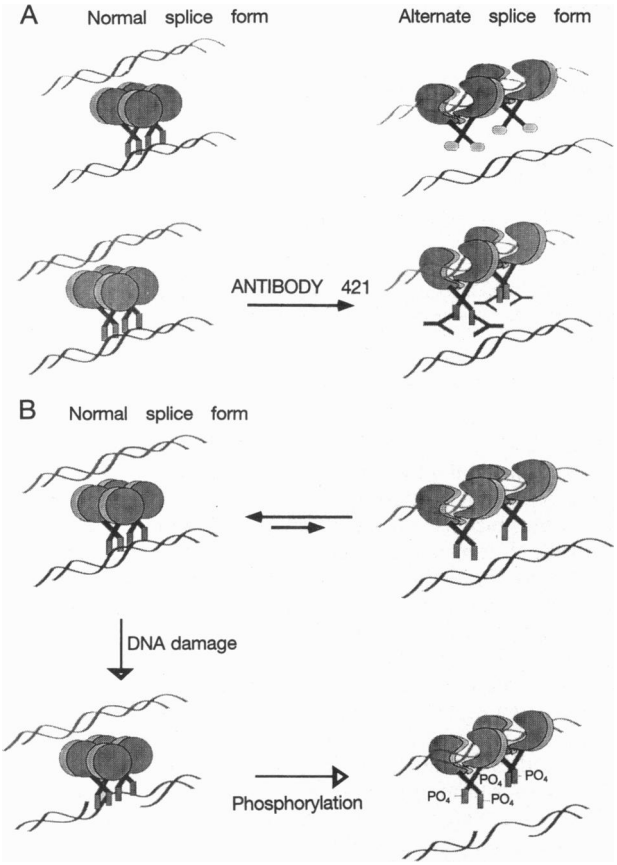

FIG. 4. Regulation of sequence-specific DNA binding by the carboxyl terminus of p53. (*A*) Nonspecific DNA binding mediated by the carboxyl-terminal DNA-binding domain of NSp53 inhibits sequence-specific DNA binding mediated by the central DNA-binding domain. The central DNA-binding domain of p53 (depicted as a tetramer) mediates the p53 protein's recognition of its specific DNA element. A nonspecific DNA-binding activity is mediated by the extreme carboxyl terminus of NSp53. When bound to nonspecific DNA (in black below the protein complex) through the carboxyl-terminal domain, p53 is locked in a conformation that cannot bind to its specific DNA element (in grey above the protein complex). ASp53 lacks the nonspecific DNA-binding domain and exists constitutively in a form capable of binding its specific recognition element. Binding by antibody PAb 421 to the carboxyl terminus of NSp53 can inhibit nonspecific DNA binding mediated by this domain and thus activates NSp53 to adopt its specific DNA-binding conformation. (*B*) A model for the regulation of sequence-specific DNA binding by NSp53 following DNA damage. As depicted, NSp53 may exist in an equilibrium between a latent state bound to nonspecific DNA and an active form capable of binding to its specific recognition element. In normal cells, most of the p53 exists in the latent state because of the large excess of nonspecific DNA present in the cell nucleus. Evidence exists that the carboxyl-terminal nonspecific DNA-binding domain of p53 has a higher affinity for regions of single-stranded DNA or DNA termini over double-stranded DNA (S. Lee, B.E., A.J.L., and J. Griffith, unpublished observation). These substrates are similar to lesions produced following DNA damage, suggesting that the carboxyl-terminal DNA-binding domain may function to recognize DNA damage directly. Colocalization of protein kinases to sites of DNA damage is proposed to be a mechanism by which p53 protein may be activated for sequence-specific binding via phosphorylation at its carboxyl terminus.

higher ASp53 levels may be present in cells during the G_2 phase of the cell cycle (30). Therefore, studies directed toward the regulation of splice-site usage in the p53 transcript may be a fruitful avenue for investigation.

1. Fields, S. & Jang, S. K. (1990) *Science* **249,** 1046–1049.
2. Raycroft, L., Wu, H. & Lozano, G. (1990) *Science* **249,** 1049–1051.
3. O'Rourke, R. W., Miller, C. W., Kato, G. J., Simon, K. J., Chen, D.-L., Chang, C. V. & Koeffler, H. P. (1990) *Oncogene* **5,** 1829–1832.
4. Lin, J., Chen, J., Elenbaas, B. & Levine, A. J. (1994) *Genes Dev.* **8,** 1235–1246.
5. Bargonetti, J., Manfredi, J. J., Chen, X., Marshak, D. R. & Prives, C. (1994) *Genes Dev.* **7,** 2565–2574.
6. Cho, Y., Gorina, S., Jeffrey, P. D. & Pavletich, N. P. (1994) *Science* **265,** 346–355.
7. Wang, Y., Reed, M., Wang, P., Stenger, J. E., Mayr, G., Anderson, M. E., Schwedes, J. F. & Tegtmeyer, P. (1994) *Genes Dev.* **7,** 2575–2586.
8. Pavletich, N. P., Chambers, K. A. & Pabo, C. O. (1994) *Genes Dev.* **7,** 2556–2564.
9. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C. & Vogelstein, B. (1991) *Science* **252,** 1708–1711.
10. Bargonetti, J., Friedman, P. N., Kern, S. E., Vogelstein, B. & Prives, C. (1991) *Cell* **65,** 1083–1091.
11. El-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W. & Vogelstein, B. (1992) *Nat. Genet.* **1,** 45–49.
12. Zambetti, G. P., Bargonetti, J., Walker, K., Prives, C. & Levine, A. J. (1992) *Genes Dev.* **6,** 1143–1152.
13. Kern, S., Pietenpol, J. A., Thiagalingam, S., Seymour, A., Kinzler, K. & Vogelstein, B. (1992) *Science* **256,** 827–832.
14. Barak, Y., Juven, T., Haffner, R. & Oren, M. (1993) *EMBO J.* **12,** 461–468.
15. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W. & Vogelstein, B. (1993) *Cell* **75,** 817–825.
16. Funk, W. D., Pak, D. J., Karas, R. H., Wright, W. E. & Shay, J. W. (1992) *Mol. Cell. Biol.* **12,** 2866–2871.
17. Wu, X., Bayle, J. H., Olson, D. & Levine, A. J. (1993) *Genes Dev.* **7,** 1126–1132.
18. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B. & Fornace, A. J., Jr. (1992) *Cell* **71,** 587–597.
19. Milner, J., Medcalf, E. A. & Cook, A. C. (1991) *Mol. Cell. Biol.* **11,** 12–19.
20. Shaulian, E., Zauberman, A., Ginsberg, D. & Oren, M. (1992) *Mol. Cell. Biol.* **12,** 5581–5592.
21. Friedman, P., Chen, X., Bargonetti, J. & Prives, C. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3319–3323.
22. Clore, G. M., Omichinski, J. G., Sakaguchi, K., Zambrano, N., Sakamoto, H., Appella, E. & Gronenborn, A. M. (1994) *Science* **265,** 386–391.
23. Wu, L., Bayle, J. H., Elenbass, B. & Levine, A. J. (1995) *Mol. Cell. Biol.* **15,** 497–504.
24. Oberosler, P., Hloch, P., Ramsperger, U. & Stahl, H. (1993) *EMBO J.* **12,** 2389–2396.
25. Bakalkin, G., Yakovleva, T., Selivanova, G., Magnusson, K. P., Szekzly, L., Kiseleva, E., Klein, G., Terenius, L. & Wiman, K. G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 413–417.
26. Hupp, T., Meek, D., Midgley, C. A. & Lane, D. (1992) *Cell* **71,** 875–886.
27. Hupp, T. R., Meek, D. W., Midgley, C. A. & Lane, D. P. (1993) *Nucleic Acids Res.* **21,** 3167–3174.
28. Arai, N., Nomura, D., Yokota, K., Wolf, D., Brill, E., Shohat, O. & Rotter, V. (1986) *Mol. Cell. Biol.* **6,** 3232–3239.
29. Han, K. A. & Kulesz-Martin, M. F. (1992) *Nucleic Acids Res.* **20,** 1979–1981.
30. Kulesz-Martin, M. F., Lisafeld, B., Huang, H., Kisiel, N. D. & Lee, L. (1994) *Mol. Cell. Biol.* **14,** 1698–1708.
31. Wu, Y., Liu, Y., Lee, L., Miner, Z. & Kulesz-Martin, M. (1994) *EMBO J.* **13,** 4823–4830.
32. Lassar, A. B., Davis, R. L., Wright, W. E., Kadesch, T., Murre, C., Voronova, A., Baltimore, D. & Weintraub, H. (1991) *Cell* **66,** 305–315.
33. Shaulsky, G., Goldfinger, N., Ben-Ze'ev, A. & Rotter, V. (1990) *Mol. Cell. Biol.* **10,** 6565–6577.
34. Wade-Evans, A. & Jenkins, J. R. (1985) *EMBO J.* **4,** 699–706.
35. Harlow, E. & Lane, D. P. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
36. Harvey, D. & Levine, A. J. (1991) *Genes Dev.* **5,** 2375–2385.
37. Yewdell, J., Gannon, J. V. & Lane, D. P. (1986) *J. Virol.* **59,** 444–452.
38. Harlow, E., Crawford, L. V., Pim, D. C. & Williamson, N. M. (1981) *J. Virol.* **39,** 861–869.
39. Dulic, V., Kaufmann, W. K., Wilson, S. J., Tlsty, T. D., Lees, E., Harper, J. W., Elledge, S. J. & Reed, S. I. (1994) *Cell* **76,** 1013.
40. Perry, M. E., Piette, J., Zawadzki, J., Harvey, D. & Levine, A. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11623–11627.
41. El-Deiry, W. S., Harper, J. W., O'Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., Want, Y., Wiman, K. G., Mercer, W. E., Kastan, M. B., Kohn, K. W., Elledge, S. J., Kinzler, K. W. & Vogelstein, B. (1994) *Cancer Res.* **54,** 1169–1174.
42. Meek, D. W., Simon, S., Kikkawa, U. & Eckhart, W. (1990) *EMBO J.* **9,** 3253–3260.